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A new fiber-optic sensor (optrode) for humidity has been developed. The sensor utilizes a fluorescent dye entrapped within a perfluorinated ionomer matrix. The fluorescence intensity increases strongly and linearly with increasing water-vapor partial pressure even though the lifetime of the optrode is approximately 1 second and the presence of CO_2 has no detectable effect on the determination of humidity. Apparently, the immobilized fluorescent dye, rhodamine 6G, associates with water to form a complex with a higher absorptivity. The dependence of fluorescence lifetime on emission wavelength revealed the co-existence of multiple excited states for the water-dye system.

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A NEW FLUORESCENCE SENSOR FOR QUANTIFICATION OF ATMOSPHERIC HUMIDITY

by

Chu Zhu, Frank V. Bright, Wayde A. Wyatt and Gary M. Hieftje

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Bloomington, Indiana 47405

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INTRODUCTION

In recent years, fiber-optic sensors have attracted more and more chemists because of their remote-analysis capability (including in-vivo applications), high sensitivity, ruggedness, and compactness. Sensor-based analysis is possible in hostile (i.e., radioactive, high temperature, etc.) environments where normal sampling methods present a hazard to both the laboratory personnel and measurement instrumentation. To date, fiber-optic sensors have been developed to measure a variety of chemical species and physical properties, including metal ions (1), partial pressure of O_2 in blood (2), pH (3,4), and humidity (5,6).

The developments in humidity sensors can be divided into two categories of devices. The first is based upon the response of electronic devices (e.g., moisture-sensitive capacitors, resistors), which are commercially available. These electronic sensors are temperature-sensitive, so temperature compensation is necessary. The second approach is based upon a change of spectral response and arises because certain species complex with H_2O and change color. Cobalt(II) chloride combined with silica is often qualitatively used as a humidity indicator. A fiber-optic sensor for humidity measurements based upon absorption spectra has been described recently by Russell and Fletcher (5). In their device, a cobalt chloride/gelatin film is immobilized on a silica optical fiber. Ballantine and Wohltjen (6) described an optical waveguide humidity sensor that employed the same colorimetric reagent.

In the present paper, we describe a new fluorescent fiber-optic sensor for humidity quantification. The humidity-sensitive material consists of rhodamine 6G incorporated into a perfluorinated polymer

matrix. In contrast to the cobalt/gelatin film approach, our device exhibits a much faster response time and provides an increase in fluorescence intensity that is linear with H_2O partial pressure. The performance of the new sensor appears to arise from a complex that is formed between water and the immobilized dye. Through examination of absorption spectra and fluorescence lifetimes, it seems that response is due to an increase in absorption of incident radiation and not to a change in dye quantum efficiency.

EXPERIMENTAL

The moisture-sensitive film was prepared from a solution of Nafion (catalog #: 27,470-4 Aldrich Chemical Co.,), rhodamine 6G (Exciton Co.) and ethanol. First, a 1.0 mM ethanolic solution of rhodamine 6G was prepared and mixed with an equal amount of the stock Nafion solution. After the solvent evaporated naturally at room temperature, the resulting film has a thickness of 25 μm and contained dye at a concentration of about 1%.

When the optrode was exposed to relatively strong laser radiation (greater than 200 mW) for several hours, fluorescence intensity was found to decrease with time. This loss of intensity was believed to originate from photodegradation of the dye. Importantly, because the laser power during the measurement process was far lower, the effect of photodegradation of 6G was not detectable.

Controlled environments containing known H_2O partial pressures were produced in a thermally controlled water-containing cuvette. A mode-locked argon-ion laser (Spectra Physics Inc. Model 171 laser, model 342 mode locker, and model 452 mode-locker driver) was used as the

excitation source. The laser energy input to the optical fiber was 50 mW for steady-state fluorescence measurement and 200 mW for lifetime determination, both at the 514.5 nm line.

The experimental system for the determination of fluorescence lifetimes (shown in Figure 1) is similar to that described by Bright et al. (7). The laser beam was mechanically chopped and focused into one end of the optical fiber (200 μm core, UV grade, Galileo Inc.). The fluorescence signal measured by a photomultiplier tube (PMT) is sent to a sampling oscilloscope which is triggered by the synchronous output of the mode-locker driver. The output of the oscilloscope is then sent to a lock-in amplifier, which is referenced to the mechanical chopping frequency. The lock-in amplifier reduces additive noise introduced after the chopper. Lastly, the output from the lock-in amplifier is sent to a computer (MINC 11/23) which also controls the scan rate of the oscilloscope time base. The UV-visible spectra of the optrode were measured with an HP 8450A UV/VIS Spectrophotometer at a spectral resolution of 1 nm.

RESULTS AND DISCUSSION

Fluorescence Intensity

Fluorescence emission spectra of the optrode in different gaseous environments are shown in Figure 2. When the optrode was bathed with dry nitrogen, the fluorescence emission spectrum exhibited peaks at 548 and 570 nm. Because these bands had similar intensities and overlapped, the emission spectrum appears to possess a plateau. However, the fluorescence intensity at 548 nm increased and that at 570 nm decreased when the partial pressure of water vapor was raised. Therefore, 548 nm

was taken as the analytical wavelength. The variation of fluorescence intensity with partial pressure of water vapor is linear, as shown in Figure 3. The correlation coefficient of the best-fit line is 0.963.

Several situations can produce multiple peaks in fluorescence emission spectra. One is the transition from the lowest excited state to different vibrational levels of the ground electronic state. Another is the transition from different excited electronic states to the ground state. In most cases, the former is dominant because the rate of internal conversion is very fast (10^{-12}s). Also, if more than one component exists in the sample, multiple emission peaks can be attributed to them.

Interactions between fluorophores and solvents also can induce a shift in fluorescence emission spectra. This shift is related to the refractive index (n) and dielectric constant (ϵ) of the solvent, and to the specific chemical properties of the fluorophores and solvent (8). The absorption spectra of the optrode (Figure 4) show that the wavelength of maximum absorption shifted from 470 nm to 520 nm when the partial pressure of water vapor increased.

We attribute this shift to the formation of a complex between H_2O and rhodamine 6G (R6G). The matrix of the optrode used in this study is an ionic polymer (Nafion). Because of its high polarity, the polymer very easily adsorbs water so a thin layer of water forms on the optrode surface. The fluorophores then form unidentified complex compounds with this adsorbed water and cause a red shift in the absorption spectrum of the optrode.

The fluorescence emission intensity depends upon two factors. One is the rate and extent of non-radiative transitions, including

vibrational relaxation, solvent relaxation and other deactivating processes. When the "quencher" concentration is increased, the fluorescence intensity will be lowered and lifetime shortened. The relationship between the intensity of fluorescence and "quencher" concentration has been treated quantitatively (8). Another, often neglected, factor that affects fluorescence intensity is excitation efficiency. If excitation efficiency is enhanced, fluorescence will increase proportionally because more excited molecules are produced.

Figure 4 shows that the absorbance of the optrode around 520 nm increases as the partial pressure of water vapor is raised. We believe this band is attributable to the complex of R6G and water. The excitation wavelength in the present optrode investigation was 514.5 nm. The enhancement of absorbance of the optrode at 520 nm means that the complex can absorb more laser energy and subsequently produce more excited-state molecules. This is apparently the reason that the fluorescence intensity increases with the partial pressure of water vapor.

Fluorescence Lifetime

Usually, the presence of a solvent will shorten a fluorescence lifetime and proportionately diminish fluorescence intensity. Figure 5 demonstrates that the lifetime was indeed shortened with an increase in the partial pressure of water vapor. Although this same trend occurred over the whole wavelength region in which the fluorescence lifetimes were measured (from 540-600 nm), the specific lifetime depended on the monitored emission wavelength. This dependence will be discussed later. It at first seems peculiar that the presence of water vapor caused the

fluorescence intensity to increase while the fluorescence lifetime decreased.

The decrease of fluorescence lifetime with an increase in the partial pressure of water vapor could be attributed to the quenching effect of H_2O. Such quenching processes are complicated and are often divided into two categories: dyanmic and static. Dynamic quenching is caused by the collision and energy transfer between quenchers and fluorophores. In contrast, static quenching is produced by the formation of a nonfluorescent adduct between the ground-state fluorophores and quenchers. Both quenching processes can be described by the Stern-Volmer equation and distinguished by lifetime measurement. For static quenching, $\tau_o/\tau = 1$ and for dynamic quenching, $\tau_o/\tau = F_o/F$ (8), where F and F_o are the fluorescence intensities and τ and τ_o the fluorescence lifetimes in the presence and absence of quenchers, respectively. From Figure 5, it is obvious that $\tau_o/\tau \neq 1$, so the quenching process is not static. However, because $\tau_o/\tau \neq F_o/F$ ($\tau_o/\tau > 1$ while $F_o/F < 1$) the quenching process cannot be described as a normal dynamic quenching process either. This apparent contradiction occurs because the fluorescent complex formed between R-6G and H_2O has a higher absorbance but shorter lifetime than the immobilized R6G itself (see Figure 4).

It was mentioned that the measured fluorescence lifetime depends on the monitored emission wavelength. This situation can occur only when more than one fluorescing species is present or when the observed transitions arise from different electronically excited states, an unlikely situation for liquid or solid samples. In the present investigation, it is believed that a part of the immobilized dye forms a

complex with water; furthermore, because the absorbance increases with the partial pressure of water vapor, the complex-formation reaction is not saturated. That is, we feel there are two forms of dye in the film, one in the complex form, and the other consisting of free (unhydrated) dye molecules. This hypothesis would account for both the wavelength-dependent fluorescence lifetimes and also the change in absorption that occurs in the presence of water vapor.

Ordinarily, the formation of a complex will lower an excited energy level, so the emission spectra are broadened and shifted to the red. However, in our case, the emission spectrum of the hydrated complex compound is shifted to the blue (Figure 2). This behavior is consistent with the model illustrated in Figure 6. In this model, the energy of both the excited and ground states is lowered, but the latter to a greater extent. The transition for the complex would then occur near the short wavelength edge of the emission spectrum. If τ_c and τ_f designate the lifetimes of the excited state of the complex and unhydrated dye molecules, respectively, the apparent lifetime should be closer to τ_c when the measurement is carried out at a short wavelength, but closer to τ_f, when the measurement is performed at a long wavelength. If τ_c is not equal to τ_f, the measured lifetime will then vary with the monitored emission wavelength.

If the emission peaks caused by the different hypothesized excited states were well separated, the respective lifetimes could be measured individually. In practice, however, spectral overlap make this method impossible. Indeed, the observed dependence of fluorescence lifetime on the emission wavelength (Figure 5) implies that the decay might be a multiple exponential process and that several dye forms might co-exist.

That is, the complex might have more than one structure (e.g., it might be combined with a different number of water molecules,.

Effects of CO_2 on the Optrode

Because CO_2 is one of the components of the atmosphere and can combine with water to form H^+ and CO_3^{-2} ions, it might interfere with the humidity measurement. Therefore, the effect of CO_2 on the response of the optrode was studied. The IR spectra of the optrode, shown in Figure 7, reveal the band at 1725 cm^{-1} (C=O) to be stronger when the optrode is in air than when it is in dry nitrogen. Presumably, this change is induced by the adsorption of CO_2 on the optrode surface. Yet the UV-visible absorption and fluorescence emission spectra (including spectral shapes and intensities) of the optrode are the same in a CO_2 atmosphere as in dry N_2. That is, the presence of CO_2 has no detectable effect on the response of the optrode for humidity measurements.

Conclusions

The optrode with immobilized Rhodamine 6G is very sensitive to the presence of water vapor (P_{H_2O}). In addition, the response time is very fast. The observed changes in fluorescence intensity can be attributed to the formation of a complex between immobilized Rhodamine 6G and H_2O. The complex appears to have a higher absorbance and consequently greater excitation efficiency than the immobilized dye by itself. The fluorescence lifetime of the optrode decreases with rising P_{H_2O} in a way that cannot be described simply by the Stern-Volmer relationship. The presence of CO_2 has no direct effect on the humidity measurement.

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FIGURE CAPTIONS

Figure 1. Block diagram of experimental system for fluorescence lifetime measurements.

Figure 2. Emission spectra of the optrode in the indicated environment (excitation wavelength: 514.5 nm). N_2 refers to a dry-nitrogen atmosphere, AIR to laboratory environment, and H_2O to a location just above the water level in an open vessel.

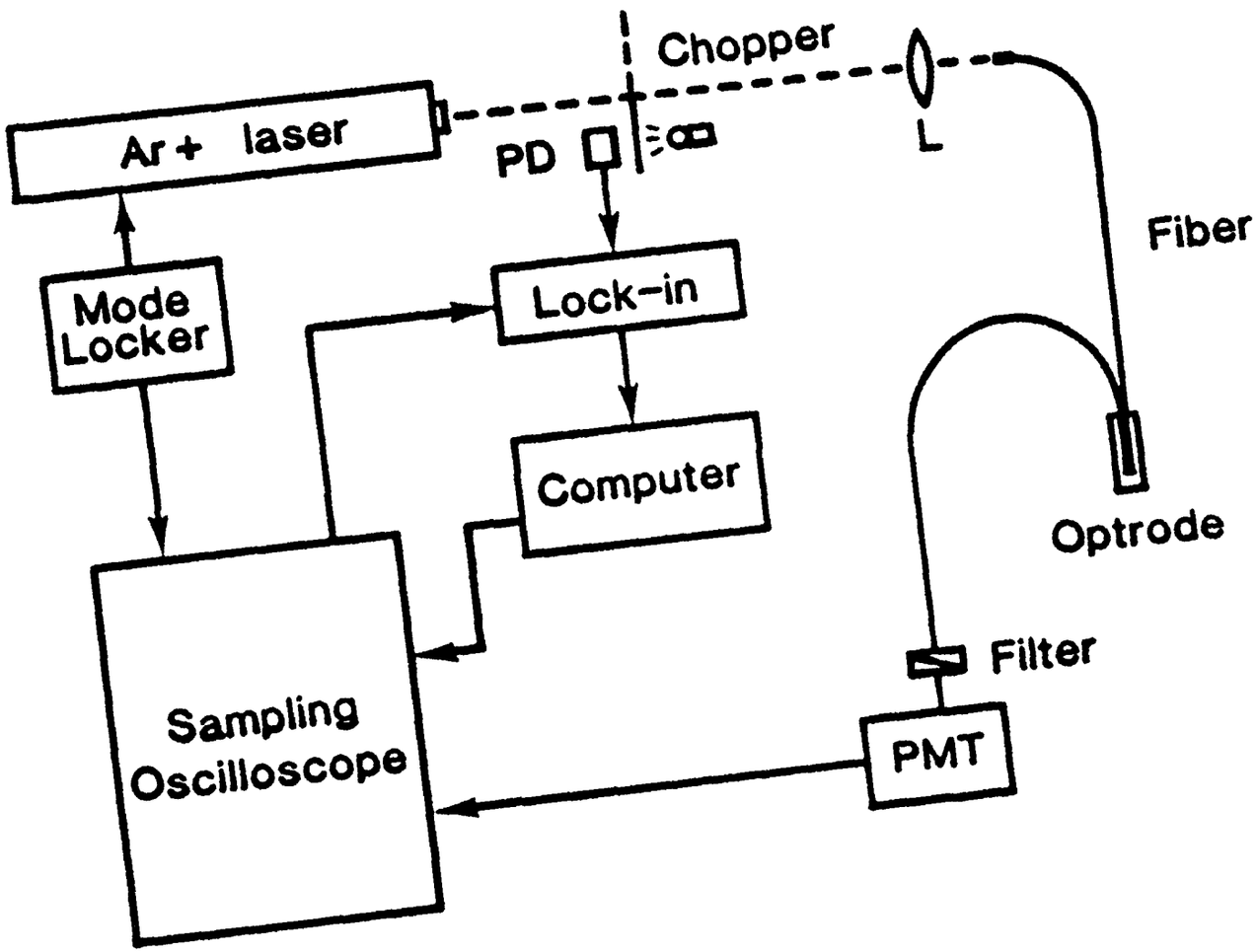
Figure 3. Calibration curve for the water partial pressure mea surement. The correlation coefficient is 0.963.

Figure 4. Absorption spectra of the optrode in the indicated atmosphere. N_2 pertains to dry nitrogen, AIR to the slightly humid laboratory environment, and H_2O to the region directly above a cell containing liquid water.

Figure 5. Fluorescence lifetime measured as a function of emission wavelength (excitation wavelength: 514.5 nm). See Figure 4 for definition of N_2, AIR, and H_2O traces.

Figure 6. Explanation of the dependence of fluorescence lifetime on the monitored emission wavelength. Note: only the first excited states are displayed.

Figure 7. IR spectra of the optrode measured in laboratory air and in dry nitrogen. Resolution: 8 cm^{-1}.


Chopper
Ar+ laser
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Fiber
Mode Locker
Lock-in
Computer
Optrode
Filter
Sampling Oscilloscope
PMT

Fig. 1

Emission spectra of the optrode

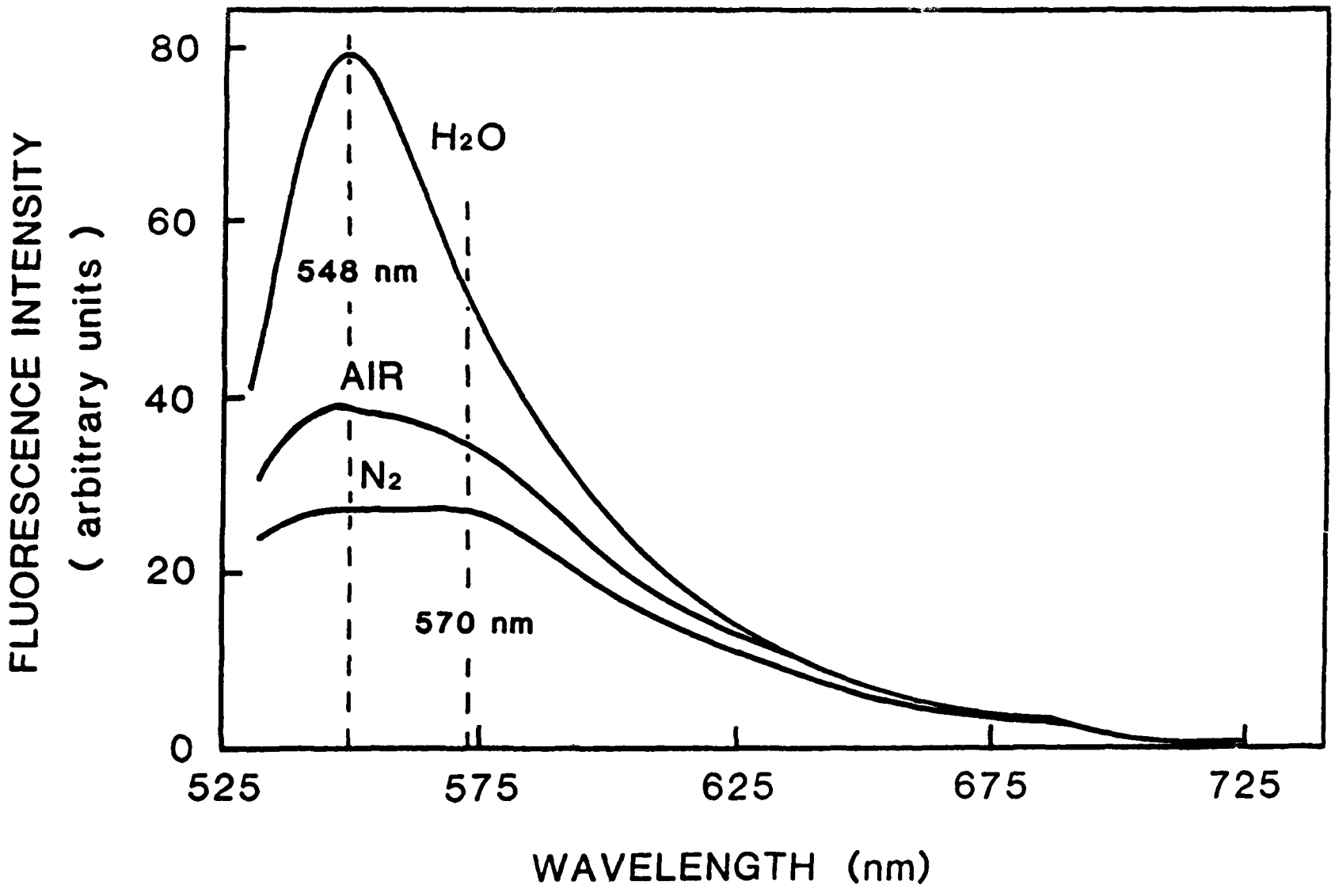

Fig 2-

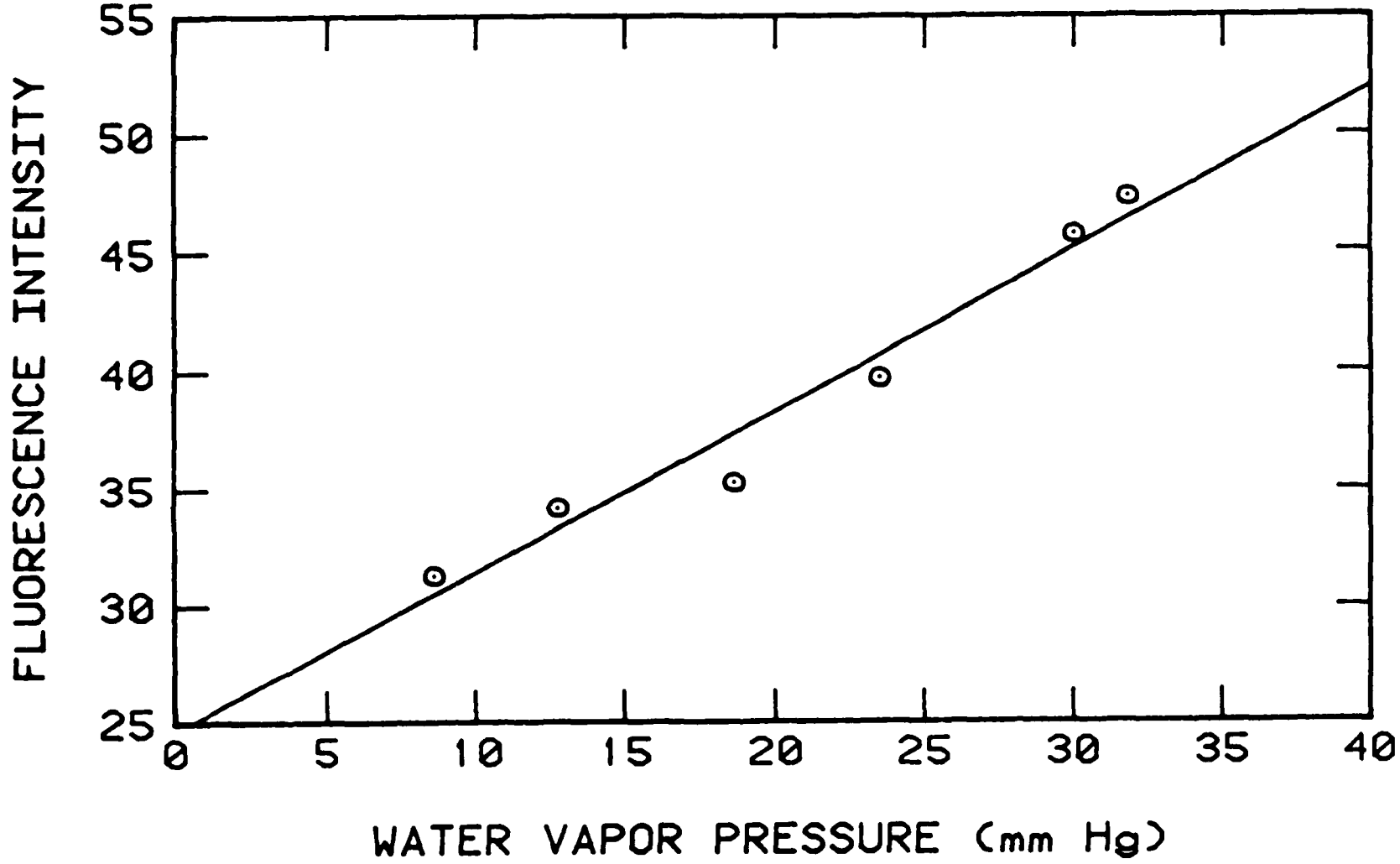
FLUORESCENCE INTENSITY
55
50
45
40
35
30
25
0
5
10
15
20
25
30
35
40
WATER VAPOR PRESSURE (mm Hg)

Fig 3

Absorption Spectra of the optrode

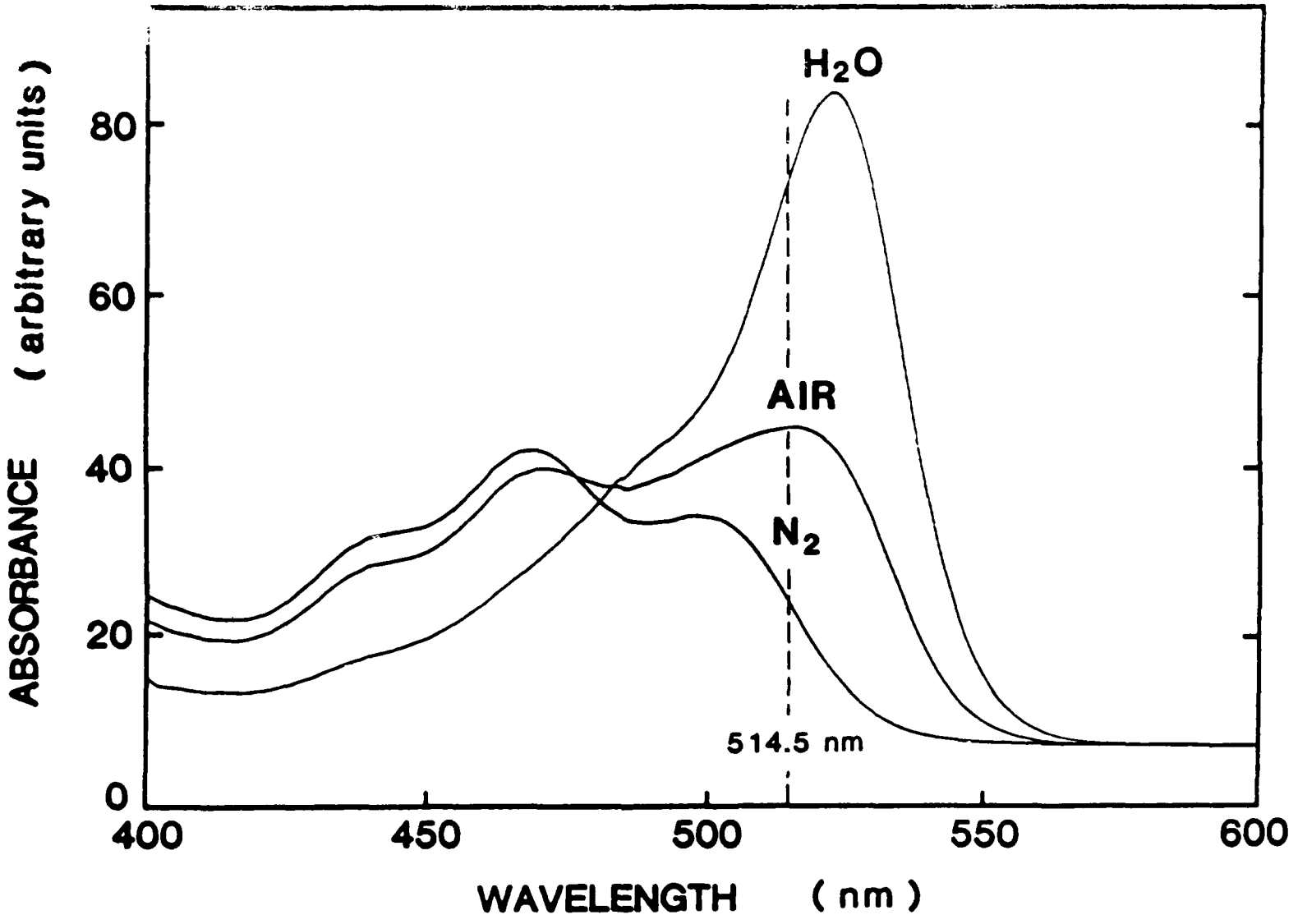

Fig 4

Fluorescence Lifetimes of the Optrode

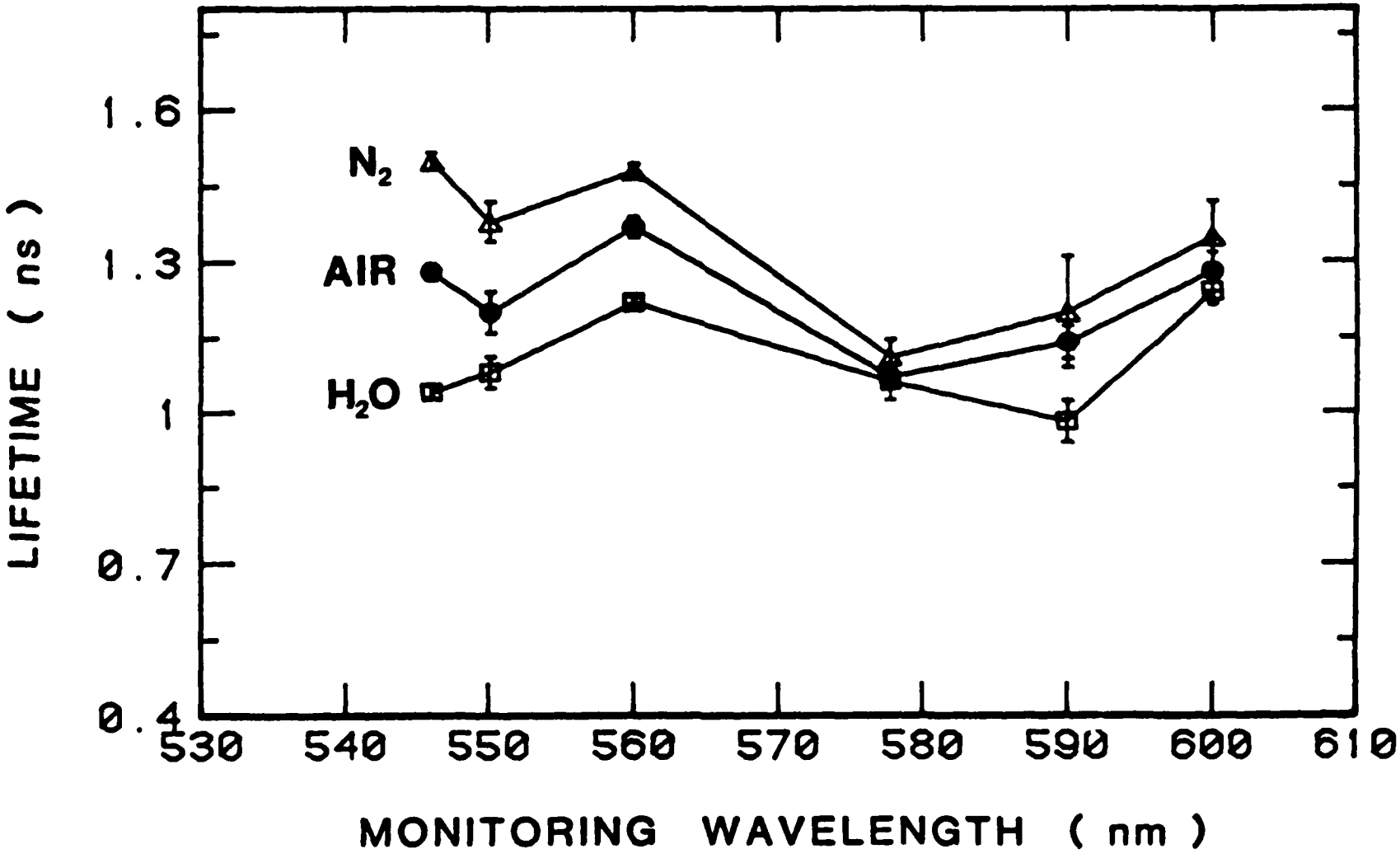

Fig 5

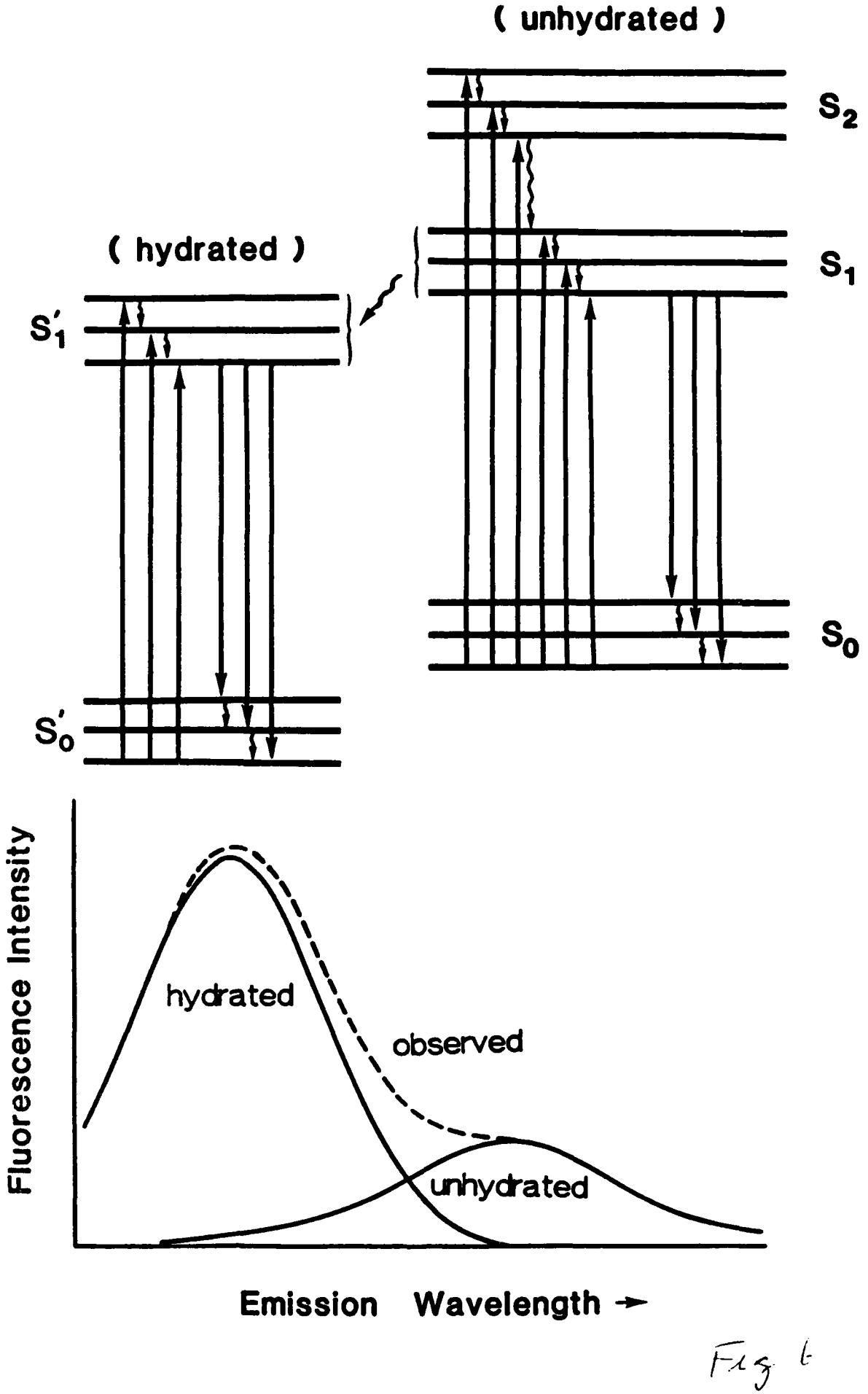
(unhydrated)
S2
S1
(hydrated)
S'1
S'0
S0
Fluorescence Intensity
hydrated
observed
unhydrated
Emission Wavelength →

Fig 6

IR Spectra of the Optrode

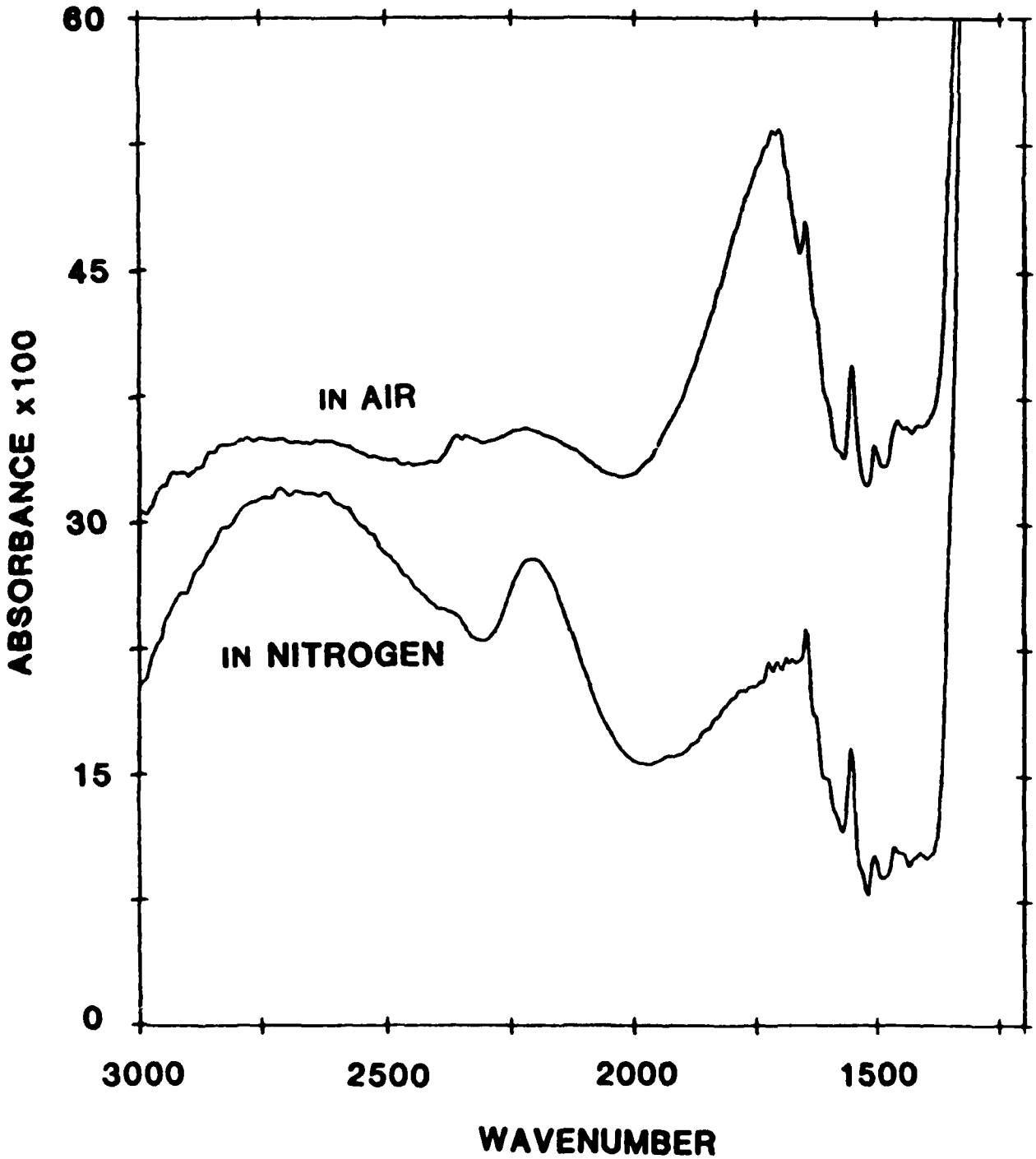

Fig. 1

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